

AB The work describes a novel approach for sustained photobiological production of H₂ gas via the reversible hydrogenase pathway in the green alga *Chlamydomonas reinhardtii*. This single-organism, two-stage H₂ production method circumvents the severe O₂ sensitivity of the reversible hydrogenase by temporally separating photosynthetic O₂ evolution and carbon accumulation (stage 1) from the consumption of cellular metabolites and concomitant H₂ production (stage 2). A transition from stage 1 to stage 2 was effected upon S deprivation of the culture, which reversibly inactivated photosystem II (PSII) and O₂ evolution. Under these conditions, oxidative respiration by the cells in the light depleted O₂ and caused anaerobiosis in the culture, which was necessary and sufficient for the induction of the reversible hydrogenase. Subsequently, sustained cellular H₂ gas production was observed in the light but not in the dark. The mechanism of H₂ production entailed protein consumption and electron transport from endogenous substrate to the cytochrome b₆-f and PSI complexes in the chloroplast thylakoids. Light absorption by PSI was required for H₂ evolution, suggesting that photoreduction of ferredoxin is followed by electron donation to the reversible hydrogenase. The latter catalyzes the reduction of protons to molecular H₂ in the chloroplast stroma.

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